Reciprocal stimulation of the two nucleotide hydrolysis reactions in the SRP(D251N)•FtsY complex is impaired to the same degree

Wild type SRP and FtsY reciprocally stimulate each other's GTPase activity in a symmetrical fashion, i.e., two GTP molecules are hydrolyzed at the same rate by SRP and FtsY in the complex (*1*). In Figure 2 of the article, we showed that mutant SRP(D251N) activates the GTPase reaction of FtsY, albeit at a much reduced rate. To test whether FtsY reciprocally activates mutant SRP(D251N), we determined the effect of FtsY on the XTP hydrolysis reaction of mutant SRP(D251N). To allow for more accurate quantitation, we performed single turnover experiments with a fixed amount of SRP(D251N) in excess over XTP and with varying amounts of FtsY. As shown in Figure S1a, the rate of XTP hydrolysis from SRP(D251N) is stimulated by increasing amount of FtsY. Using an analogous experimental set-up, the reciprocal reaction, GTP hydrolysis by FtsY, is also shown to be stimulated by increasing amounts of SRP(D251N) (Fig. S1b), confirming the results presented in the article (Fig. 2). Thus qualitatively, mutant SRP(D251N) and FtsY can act as reciprocal GTPase activating proteins for one another, mimicking the properties of wild type SRP.

However, quantitatively the ability of mutant SRP(D251N) and FtsY to activate each other is vastly compromised. The maximal XTPase rate constant with saturating FtsY (Fig. S1a), which represents the rate constant for the reaction: ^{XTP}•SRP(D251N)•FtsY•^{GTP} → XDP + P_i (k_c^{XTP}), is only 0.84 min^{-1}, 50-fold slower than the value of 42 min^{-1} observed for the reaction of the ^{GTP}•SRP•FtsY•^{GTP} complex. Similarly, the GTPase rate constant with saturating SRP(D251N) is only 0.34 min^{-1} at 10 μM FtsY (Fig. S1b). At this FtsY concentration, ~40% of the available GTP is bound by FtsY (K_d = 15 μM). The rate constant for GTP hydrolysis from the ^{XTP}•SRP(D251N)•FtsY•^{GTP} complex, therefore, calculates to 0.85 min^{-1}, consistent with the

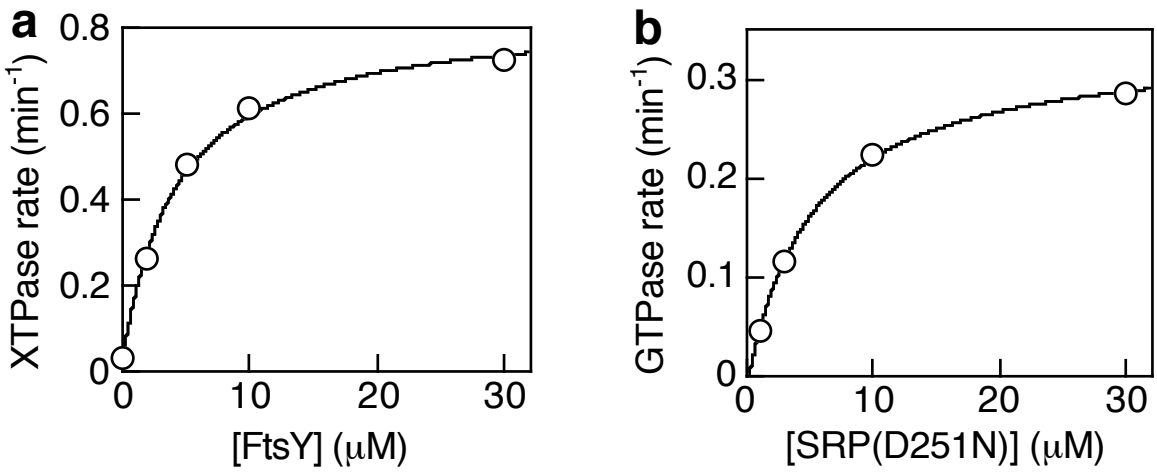
rate constant determined independently in Figure 2 in the article. This, again, is 50-fold slower than the reaction from the wild type complex and is the same, within error, as the rate of FtsY-stimulated XTPase reaction from SRP(D251N) (Fig. S1a). Thus, in the SRP(D251N)•FtsY complex both proteins are significantly less active in stimulating each other's hydrolysis reactions than in the wild type complex. The symmetry of the reaction, however, is maintained as both nucleotides are hydrolyzed at the same rate by the two respective active sites in the complex.

References

1. Powers, T., and Walter, P. (1995) Reciprocal stimulation of GTP hydrolysis by two directly interacting GTPases, *Science 269*, 1422-1424.

Supplementary Figure 1 Reciprocal GTPase and XTPase stimulation between SRP(D251N) and FtsY. **(a)** FtsY stimulates the XTPase reaction of SRP(D251N). Observed XTPase rate constant was determined in single turnover experiments with trace amounts of XTP* (< 0.1 nM), 2 μM SRP(D251N), and 50 μM GTP, as described in the Methods. The line is a fit of the data to eq 2 in the article, and gave a maximal XTPase rate constant of 0.84 min^{-1}. **(b)** SRP(D251N) stimulates the GTPase reaction of FtsY. Observed GTPase rate constant was determined in a similar setup as in part **(a)**, with trace amounts of GTP* (< 0.1 nM), 10 μM FtsY and 50 μM XTP. The line is a fit of the data to eq 1 in the article, and gave a maximal GTPase rate constant of 0.34 min^{-1}.

Figure S1


a
XTPase rate (min-1)
0.8
0.6
0.4
0.2
0
0
10
20
30
[FtsY] (μM)
b
GTPase rate (min-1)
0.3
0.2
0.1
0
0
10
20
30
[SRP(D251N)] (μM)